

4. The tilt from reclining to head up 45 degrees produced almost the same degree of responses as the tilt from reclining to standing.

5. With the exception of the tilts in the head down to reclining quadrant the responses given to any tilt were offset by approximately equal opposite responses when the tilt was reversed.

6. The initial and final positions of the subject had more effect on the degree of responses following a tilt than did the distance travelled by the subject during the tilt.

The writer wishes to acknowledge his obligations to Lieut. Harry Fried, M.C., who assisted in a large number of these tilting tests.

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LABORATORY AIDS IN THE DIAGNOSIS OF GONOCOCCAL INFECTIONS, WITH SPECIAL REFERENCE TO THE GONOCOCCUS COMPLEMENT-FIXATION TEST.

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THE present intensive campaign for the education of the general public in regard to the incidence, treatment and prevention of venereal diseases will, it is hoped, do much to lead to their early recognition, and, following upon this, and instructions as to their infectivity—which must, of necessity, form part of their treatment—great strides in their prevention are to be hoped for.

Indeed, it is now not an infrequent occurrence for those about to marry to seek information as to their freedom from venereal infection and because of this fact, and because, particularly in cases of long standing or those whose infection long antedates their visit to the doctor, the question as to their ability to infect others is a matter difficult to decide, a *resume* of the laboratory aids in the diagnosis of gonococcal infections seems timely.

In spite of the fact that the recognition of the typical case of gonorrhea is, as a rule, a matter of no difficulty, there are a number of cases of urethritis, especially in the female, where the diagnosis is not readily to be made.

A wrong positive diagnosis is an injury to the patient not readily

repaired; a wrong negative diagnosis may cause irreparable harm to the patient, his family and to the community, and every method available to avoid either error should be called upon whenever possible.

It is, of course, an obvious and admitted fact that not all cases can be recognized with absolute certainty by laboratory methods alone. There is always the latent case, the chronic case, especially in the female, in which bacteriologic evidence as to its origin is lacking; and in such cases, as in other conditions, the clinical evidence must be the deciding factor. However, in a certain number of cases specific evidence can be elicited if the proper methods are applied, and to call attention to these is the object of this paper. As always, it must be remembered that it is not the test but its interpretation which is of value.

Cases in which laboratory evidence of gonococcal infection is to be sought for may be divided into three groups: (a) Acute infections actually or suspected to be of gonorrheal origin; (b) cases in a chronic stage either with or without a history of previous acute infection; (c) cases which may be looked upon as presenting secondary manifestations of the disease, examples of which are arthritis in the male and salpingitis in the female.

Bacteriologic Diagnosis of Acute Infections. These may be accompanied by a history of diagnostic value, or this may be entirely lacking, as is usually the case in the female. In the male, as a rule, the presence of an acute, urethral discharge is preponderating evidence of gonorrhea; because, however, of the occasional case of non-specific urethritis, an error in the diagnosis of which may lead to dire results, a microscopic examination of the stained smear is indicated.

There are several points in the preparation of such specimens, minor details in themselves, perhaps, but all, in their summation, enhancing or vitiating the value of the examination.

1. The patient should not void for at least an hour previous to taking the specimen, thus avoiding flushing out of the canal.

2. The smear should be from the urethra. This is of particular importance in the female, for nothing is of less diagnostic value, except in the vulvovaginitis of children, than a hastily taken smear from the vagina. In the adult female additional preparations should be made from the cervix and so labelled.

3. The smear should not be too thick, as the difference between Gram-negative and Gram-positive organisms may be a matter of some difficulty to determine, because the thickness of the smears protects them from the action of the reagents.

4. The smears should be taken in duplicate upon microscopic slides or cover-glasses which should be allowed to dry spontaneously before being placed in contact. Nothing is more unsatisfactory than slides which have been placed together while moist, have adhered upon drying and must be pried apart for staining.

5. Douches, injections, washes and the like should not be used just prior to taking the specimens.

While the typical gonococcus is readily recognized by those familiar with its morphology, in the simple, single-stained preparation, such as Loeffler's methylene blue, the fact that many times they may not be typical in appearance and that there are other organisms which morphologically resemble them in appearance, renders the use of Gram's staining technic advisable as a routine.

Chief among the organisms morphologically resembling the gonococcus is the meningococcus, which, however, is not often found in the same localities; other organisms of more importance as being seen in urethral smears are coccoid forms of the colon bacillus, phagocytized cocci which, as a result, may stain indifferently, and the *Micrococcus catarrhalis*.

What are the criteria for the recognition of the gonococcus in the stained smear? It must be Gram-negative; it must be a diplococcus and typical bean- or kidney-shaped forms must be demonstrated in some area of the smear, and careful search should demonstrate some intracellular forms. In this connection it must be borne in mind that, in the acute discharges, the majority of the gonococci are not infrequently entirely extracellular.

These conditions, having been fulfilled in the presence of an acute discharge with or without a suspicious clinical history, suffice to render the diagnosis of gonorrheal infection justifiable.

The exact technic of Gram-staining deserves a word of mention, as it is the rock on which many stumble. There are several sources of error inherent in the method itself if minor details are not carefully attended to.

1. The gentian-violet solution made with anilin water must be relatively fresh, and even when other solutions of more stable character, such as Stirling's stain, are made use of the stain should be frequently tested with known Gram-positive organisms.

2. The film must not be too thick, as decolorization may be difficult and unsatisfactory.

3. The films should be allowed to dry spontaneously, and care should be taken to avoid overheating when fixing, as atypic staining may occur.

4. The iodine solution should be stronger than that in the original formula, the water being reduced from 300 c.c. to 100 c.c. (iodine, 1 gram; potassium iodide, 2 grams; distilled water, 100 c.c.).

5. The alcohol used for decolorizing should not be weaker than 95 per cent. to absolute and should not be allowed to act for too long a time, as Gram-positive organisms resist its action only for a reasonable period and exposure of the film to alcohol for longer than two or three minutes will give confusing results.¹

¹ M. W. Lyon (Jour. Am. Med. Assn., October 9, 1920, No. 15, lxxv, 1017) has recently advocated the use of acetone as a decolorizing agent for the Gram stain, applying it instead of alcohol for five to ten seconds. Some experience with the method would seem to bear out the claims made for it.

6. The counterstain must be in watery solution, must not be too strong nor allowed to act for too long a time, as it may, in such case, replace the gentian-violet and give false Gram-negative pictures.

A satisfactory technic follows. As a preliminary it is well at frequent and definite intervals, to test the stain and the technic with a known Gram-positive organism, such as the *Staphylococcus aureus* and a known Gram-negative organism, such as *Bacillus coli*, both of which are readily obtained.

Technic for Gram-staining. 1. Fix the dried film by passage through the flame, testing the degree of heat on the hand.

2. Cover with gentian-violet solution for one minute.

3. Wash off the gentian-violet solution with Gram's iodine solution (amended formula) and replace with another fresh portion of iodine solution, which is allowed to act for one minute.

4. Wash off the iodine solution with alcohol (95 per cent. to absolute), not with water, and flood the film with alcohol, tilting the slide to allow the alcohol to flow back and forth until no more violet color is given off. The action of the alcohol should not be prolonged for more than two or three minutes, which will be sufficient if the film is of the proper thickness.

5. Counterstain thirty seconds with a weak, watery solution of Bismarck brown, eosin (0.5 per cent.) or a very dilute solution of carbol-fuchsin (two drops to a test-tube full of water).

6. Wash in water, blot and mount.

It must be remembered, in the interpretation of the stained smear, that the fact that an organism is Gram-negative, and intracellular does not, therefore, determine it as a gonococcus; familiarity with the variations in its morphology are essential. As in all other laboratory examinations, in the final analysis the value of the procedure is in direct proportion to the degree with which it is correlated with the clinical findings; in a word, it is the interpretation which counts.

Summing up: For the identification of the gonococcus in the stained smear, the following conditions must be fulfilled: (a) The smear must be properly and intelligently taken and must not be too thick; (b) the staining technic must be properly controlled; (c) the observer must be familiar with the microscopic appearance of the gonococcus and allied organisms and thoroughly capable of interpreting what he sees in terms of its relations to the patient.

These premises being fulfilled and the findings correlated with the clinical evidence the bacteriologic diagnosis of acute gonorrheal infections may be made with a fair degree of certitude.

In the comparatively infrequent cases of gonococcus bacteremia cultures are required, the preparation and study of which require a trained bacteriologist, so that they are merely mentioned here.

Bacteriologic Diagnosis of Chronic Infections. It is in cases of this group—those in which the history of a previous acute attack is not infrequently wanting and in which the clinical evidence is not

always sufficiently clear to permit of a definite diagnosis—that the laboratory is most frequently called upon to make a decision.

Here, also, we may place those individuals who, admitting a previous infection, but being without symptoms, are desirous of obtaining evidence as to their freedom from foci which might prove a source of infection to others.

We may adopt any or all of three procedures: (a) Microscopic examination of stained films from selected areas; (b) the production of an acute discharge in which gonococci may be found or the production of an acute, focal reaction; or (c) the use of the gonococcus complement-fixation test.

The efficacy of the first of these depends entirely upon the locality from which and the method by which the film is taken, for, in the majority of cases, the success or failure of the examination as to decisiveness will stand or fall upon the character of the film examined.

It is necessary to keep in mind the lurking places of the gonococcus and to remember that it may be present in these places without the coincident presence of any discharge. In the male these localities are: (a) The follicles of the urethra; (b) Cowper's glands opening into the bulbous portion of the urethra; (c) the prostate; (d) the seminal vesicles; (e) rarely in the bladder.

It is necessary, therefore, for films to be made and examined from the following: (1) Material expressed by milking the urethra; (2) material obtained by the introduction of a sterile swab into the urethra; (3) from the material obtained by prostatic massage; and (4) from the centrifugalized sediment of an ounce or so of urine passed at the time of the examination.

In the female the gonococcus may persist in: (1) The urethra and the urethral ducts; (2) Bartholin's glands; (3) the cervix; (4) the uterus and Fallopian tubes.

Smears must therefore be made from material secured by milking the urethra or the introduction of a sterile swab, from material expressed from Bartholin's glands, secretion from the cervix, or, rarely, from the uterine cavity.

It must be remembered that even these procedures may fail, for the gonococcus may still be present in other localities than those examined, as in the Fallopian tubes, or, when present, may be buried in the deeper cell layers and not liberated at the time of examination. Under these circumstances an attempt may be made to produce a focal reaction by the administration of gonococcus vaccine, which may be manifested by the production of an acute discharge containing the gonococcus or by pain and tenderness in areas of metastatic gonococcal infection. Acute discharges may be produced by the use of irritants, such as silver nitrate solution.

Several observers, notably Irons,² Bruck,³ and Reiter,⁴ have

² Jour. Infect. Dis., 1908, v, 279.

³ Deutsch. med. Wchnschr., 1909, xxxii, 470.

⁴ Ztschr. f. Geburtsh. u. Kinderh., 1911, lviii, 571.

reported upon the occurrence of focal reactions following the use of vaccines, but there is an element of danger in the procedure in that a latent infection may be converted into an acute one not readily controlled.

It may be noted in passing that Irons⁵ has reported upon the production of cutaneous reactions, similar to those obtained in the von Pirquet test, occurring with gonococcal extracts; but as the cutaneous sensibility of various individuals seems to vary, and, indeed, the same individual may show extreme variations—possibly due to varying amounts of bacterial protein in the extracts used—the test is limited in value and not generally used.

The Gonococcus Complement-fixation Test. Depending, as does the Wassermann, upon the absorption or fixation of complement, the gonococcus complement-fixation test differs from it in being a true biologic reaction in that the antigen is a true antigen composed of suspensions or extracts of the causative organism.

Müller and Oppenheim⁶ were the first to report the occurrence of the reaction, further reports later emanating from Bruck⁷ and Meakins,⁸ who published the first American report in 1907. The work of Teague and Torrey,⁹ Torrey,¹⁰ Wollstein,¹¹ Watabiki,¹² Harrison,¹³ Ower,¹⁴ and particularly that of Schwartz and Schwartz and McNeill,^{15 16 17} was largely instrumental in placing the test on a practical basis, and numerous reports have now been made upon its specificity and value in determining absolute cure.

Particular advantages of this method of testing for the presence of gonococcal infection are the fact that the specimen is easily taken and the patient is not thereby informed as to the nature of the examination.

It is not, however, as widely used as it ought to be, because, owing to the small amount of antibody produced by a localized gonococcal infection, the reaction is generally weak and may often be lacking, and the performance of the test requires the highest skill and the closest attention to details, especially as concerned with the preparation and standardization of the antigen.

It is not the purpose of this review to touch upon the technic of this reaction, as it properly belongs to the domain of the serologist, with special training in this reaction; it is rather our purpose here to consider its limitations and its interpretation and the value which may be placed upon it.

⁵ Jour. Am. Med. Assn., 1912, lviii, 931.

⁶ Wien. klin. Wehnschr., 1906, xix, 894.

⁷ Deutsch. med. Wehnschr., 1906, lxx, 36.

⁸ Johns Hopkins Med. Bull., 1907, xviii, 255.

⁹ Jour. Med. Research, 1907, xvii, 223.

¹¹ Wollstein: Jour. Exp. Med., 1907, ix, 588.

¹² Jour. Infect. Dis., 1910, vii, 159.

¹³ Jour. Royal Army Med. Corps, London, 1914, xxii, 125.

¹⁴ Jour. Canadian Med. Assn., 1916, N. S., iv, 1074.

¹⁵ Schwartz and McNeill: AM. JOUR. MED. SC., 1911, cxli, 693.

¹⁶ Ibid., 1912, cliv, 815.

¹⁰ Ibid., 1910, xxii, 95.

¹⁷ Ibid., 1912, cliv, 369.

A positive reaction is of far greater value than a negative, which again emphasizes the necessity for the proper interpretation of laboratory procedures. While non-specific reactions have been reported¹⁸ the technic by which they were obtained, particularly as to the antigens used, is not stated in detail and is concomitant with the early days of the reaction, and the majority of observers are in accord in holding that a positive reaction may be looked upon as evidence of an active focus of gonococcal infection.^{19 20 21}

Factors influencing the interpretation and reliability of the reaction may be summed up as follows:

1. Because of the small amount of antibody produced, the reaction, when positive, is weaker than the Wassermann and may be indefinite.
 2. It is frequently absent in acute, uncomplicated cases,²² and may not appear earlier than six weeks after the onset.²³
 3. In acute exacerbations of a chronic urethritis the reaction is positive in about 80 per cent. of cases; in ordinary chronic urethritis with mild prostatic involvement, the reaction is positive in 30 to 40 per cent. of cases.²⁴
 4. The occurrence of an acute complication usually gives rise to a positive reaction.
 5. A positive reaction may persist for several weeks after a clinical cure, usually lasting for two or three weeks. If obtained later than that a focus of active infection is probably present.²⁵
 6. In women the reaction is positive, as a rule, only when the infection reaches the cervical canal. According to Kolmer,²⁶ in children, positive reactions occur in acute vulvovaginitis, thus evidencing that either the infection is more severe with the consequent production of more antibodies or that the cervical canal is frequently involved.
 7. The reaction is positive in about 60 per cent. of pyosalpinx cases.
 8. In cases of gonorrheal arthritis the reaction is positive in from 80 per cent. to 100 per cent.
 9. The administration of gonococcus vaccine or antigenococcic serum may give rise to a positive reaction which may persist for six to twelve weeks.
 10. The reaction has a greater positive than negative value.
- It is thus seen there is wide field of usefulness for this test which it is to be hoped, will come into more frequent and common use in the survey of gonococcal infections.

¹⁸ Uhle and Mackinney: *New York Med. Jour.*, 1915, cii, 737.

¹⁹ Thomas and Ivy: *Arch. Int. Med.*, 1914, xiii, 737.

²⁰ Thomas, Ivy and Beardsall: *Surg., Gynec. and Obst.*, 1914, xix, 390.

²¹ Kolmer: *Infection, Immunity and Specific Therapy*, 1915, 1st ed., 482 et seq.

²² Thomas and Ivy: *Loc. cit.*

²³ Kolmer: *Infection, Immunity and Specific Therapy*, 1915, 1st ed., 482 et seq.

²⁴ *Ibid.*

²⁵ *Ibid.*

²⁶ *Ibid.*